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Sample preparation for single molecule localisation microscopy and imaging.

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We use this protocol and it's working

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Abstract

This protocol describes the sample preparation to perform SMLM on midbrain dopaminergic neurons using phalloidin and a DNA aptamer in a combination of STORM and DNA-PAINT.

It also describes the experimental set up on the microscopy with the image acquisition details.



Fixation

22m

- 1 For single molecule localisation microscopy (SMLM), neurons were grown on glass coated ibidi chambers.
- 2 Once neurons reached the desired age, they were washed once in PBS
- 3 They are then fixed for  00:15:00 in 4% paraformaldehyde + 0.1% glutaraldehyde (both from Electron Microscopy Services) in PBS at room temperature. 15m
- 4 The neurons were then reduced in 0.1% sodium borohydride (Sigma) in PBS for  00:07:00 at room temperature. 7m

Labelling cells

3h 20m

- 5 To label cells with the DNA-aptamer and phalloidin, after fixation and PBS washes, cells were permeabilised with 0.25% triton X-100 in PBS for  00:10:00 at room temperature 10m
- 6 The cells were then blocked in blocking solution (0.1% triton X-100, 10% normal goat serum (Abcam), 10% salmon sperm DNA (Thermo Fisher Scientific)) in PBS for  02:00:00 at room temperature 2h
- 7 The samples were then incubated with 100 nM of the DNA-aptamer made up in the blocking solution at  4 °C overnight.

Note

Aptamer binds to beta-sheet rich structures including protein aggregates. The sequence of the aptamer is: GCCTGTGGTGTGGGGCGGGTGC GTTATACATCTA (ATD Bio).

- 8 After incubation, cells were washed 1x in PBS



9 They are then incubated with phalloidin-647 (1:400) (Thermo Fisher Scientific) made up in the blocking solution for  01:00:00 at room temperature.

1h

10 Cells were then washed 1x in PBS and either imaged, or incubated with DAPI (1:10000) in PBS for  00:10:00 at room temperature followed by 2x PBS washes before imaging.

10m

Labelling aggregates in cell lysate

1h 15m

11 Cells were lysed mechanically in PBS before being centrifuged at

 3600 x g, 4°C, 00:05:00 .

5m

12 The supernatant was collected and the protein concentration was quantified using the BCA Protein Assay Kit (Thermo Fisher Scientific).

13 22 × 40 mm, 1 mm thick glass slides, were cleaned with an argon plasma for  01:00:00 before 22×22 gaskets (Bio-Rad) were affixed to the surface to create a well.

1h

14 Cell lysate was diluted 1 in 10 with filtered PBS (0.02 µm) and added to the glass slides

15 100 nM DNA-aptamer was added to the glass slide and incubated with lysate for

 00:10:00 .

10m

Note

Aptamer sequence: GCCTGTGGTGTGGGGCGGGTGCGTTACCACCACCACCACCA
(ATD Bio)

16 Sample was then washed off with filtered PBS three times before imaging

Microscopy imaging

17 SMLM was performed on a Nanoimager super-resolution microscope (Oxford Nanoimaging Ltd) equipped with an Olympus 1.4 NA 100x oil immersion super

apochromatic objective.

- 18 To ensure efficient stochastic blinking for STORM (AF647-tagged phalloidin), the samples were incubated with a blinking induction buffer (B cubed, ONI).
- 19 Separately to this, AD-PAINT was also employed which relies on the addition of an imaging oligonucleotide strand

to the buffer. 1 nM of the imaging strand was added to the B cubed buffer before imaging.

Note

sequence: CCAGATGTAT-CY3B

- 20 The laser illumination angle was set to 51° for all imaging leading to total internal reflection fluorescence (TIRF).
- 21 AF647-tagged phalloidin was first imaged for 4000-8000 frames using the 640 nm laser (80% power). After this, 4000-5000 frames at 30% power for the 561 nm laser was used to image and super-resolve the aptamer. Both were recorded at a frame-rate of 50 ms.
- 22 For imaging aggregates in neuronal lysate using AD-PAINT, 2 nM of the imaging strand was added. Images were acquired on Oxford Nanoimager at 20 frames s^{-1} for 8000 frames (20% 635 nm laser power, TIRF).

Note

sequence: GGTGGT-ATTO 655